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Telomere DNA Content in Prostate Biopsies Predicts Early Rise in Prostate Specific Antigen Following Radical Prostatectomy for Prostate Cancer

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Abstract

Objective—To determine whether measurement of telomere DNA content (TC) in prostate biopsy tissue predicts PSA recurrence in men after undergoing radical prostatectomy for prostate cancer.

Methods—Slot blot titration assay was used to quantitate TC in archived diagnostic prostate needle biopsy specimens for subjects ($n=103$) diagnosed with prostate cancer and who subsequently underwent radical prostatectomy between 1993 and 1997. TC was compared to the clinical outcome measure, PSA recurrence, defined as an increase in PSA ≥ 0.2 ng/mL on two or more consecutive measurements post-prostatectomy, observed retrospectively, for a mean follow-up period of 114 months (range 1–165).

Results—In the cohort, 46 subjects had a PSA recurrence. In a univariate Cox proportional hazards model, low TC (<0.3 of standard) demonstrated a significant risk for PSA recurrence (HR=1.94; 95% CI:1.02–3.69, $p=0.04$). In a subset analysis of men with biopsy Gleason sum ≤ 6 ($n=63$; 25 recurrences), a univariate Cox proportional hazards model demonstrated low TC had a greater risk of PSA recurrence (HR=4.53; 95% CI:2.00–10.2, $p<0.01$). In a multivariate Cox proportional hazards model, low TC was also significantly associated with PSA recurrence in this subset after controlling for pre-operative PSA levels (HR=6.62; 95% CI:2.69–16.3, $p<0.01$).

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Conclusions—Low TC measured in prostate biopsy tissue predicts early likelihood of post-prostatectomy PSA recurrence in a retrospective analysis and in men with biopsy Gleason sum ≤ 6 disease is also independent of pre-operative PSA level.

Keywords

prostate adenocarcinoma; genomic instability; prognosis; telomere; PSA recurrence

INTRODUCTION

In the era of prostate-specific antigen (PSA) testing, there has been a steady decrease in the number of men presenting with advanced disease compared with an increased number of localized prostate cancers detected¹. This shift to increased localized disease detection has caused some confusion and debate over appropriate screening and management strategies, as well as demonstrating a need to develop more refined techniques for determining the prognosis of prostate adenocarcinoma. A number of pretreatment prognostic tools may help predict disease outcome for patients with localized prostate cancer. Some of these include: pre-operative PSA, tumor grade by Gleason sum at biopsy and clinical stage. Pathological stage, the most reliable predictor of tumor progression, recurrence and overall disease outcome, is only available if a patient chooses to undergo radical prostatectomy. Several limiting factors surrounding the use of staging and common clinical tumor markers argue for the development of more reliable prognostic markers prior to therapeutic intervention²⁻⁴. To meet this need, efforts have focused on development of molecular markers available at the time of biopsy.

It is well established that genomic instability occurs in virtually all cancers, including prostate cancer⁵. Telomere dysfunction is one mechanism that generates genomic instability^{6, 7}. Recently, our laboratory demonstrated that a decrease in relative telomere DNA content (TC), a proxy for telomere length^{8, 9}, independently predicts prostate cancer recurrence^{10, 11}. Additionally, we observed that TC in tumor tissue correlated to matched, histologically normal adjacent tissue suggesting the presence of a “field effect”, or “field cancerization” in diseased prostates¹⁰. Therefore, measurement of TC in prostate biopsy tissue, regardless of the tumor content, could potentially give important prognostic information prior to undergoing invasive treatments. The purpose of the present study is to determine retrospectively whether TC measured in prostate biopsy tissue predicts PSA recurrence in men treated by radical prostatectomy.

MATERIAL AND METHODS

Study population

This study was approved by the University of New Mexico Human Research Review Committee and the Veteran’s Administration Research and Development Committees. All men who underwent standard clinical evaluation and treatment for prostate cancer with a perineal or retropubic radical prostatectomy without first receiving neoadjuvant radiation or hormone therapy from 1993 through 1997 at the Veteran’s Affairs Medical Center (VAMC) in Albuquerque, NM, were identified retrospectively in the electronic medical record database. Of the 125 subjects identified, men were excluded who: (i) did not have a diagnostic biopsy performed at the VAMC ($n=10$), (ii) were diagnosed by transurethral resection of the prostate ($n=5$), (iii) were determined to have pathological Tx disease ($n=3$), or (iv) were missing archived biopsy core tissue ($n=4$), leaving a total of 103 subjects. All subjects had prostate adenocarcinoma pathology at the time of pathological evaluation of the prostate biopsy.

Clinical data

Clinical data, individually reviewed and abstracted, included: age at diagnosis, race/ethnicity, pre- and post-treatment PSA measurements (ng/mL), biopsy Gleason sum according to the original clinical pathology biopsy report, pathological stage (expressed as a binary variable defined as “organ confined” if pT2c or less), grade (“pathological Gleason sum”), surgical margin status, neoadjuvant and adjuvant prostate cancer specific treatments, and cause of death, where applicable. The date of the radical prostatectomy used as the follow-up period starting point and the most recent dated clinical record or date of death was used to define the follow-up end-point. In this study, we defined PSA recurrence to be a post-radical prostatectomy PSA ≥ 0.2 ng/mL on at least 2 consecutive tests after their radical prostatectomy or if they underwent adjuvant treatment within one year of radical prostatectomy despite no PSA elevation. The laboratory determining the TC of the biopsy tissues and the clinical data abstractors were blinded until all the data were independently collected and confirmed.

Determination of telomere DNA content (TC)

100 μ m of prostate biopsy core tissue was sampled in serial 25 μ m sections from archived paraffin blocks for each subject. Prostate cancer tissue was not microdissected from the core. TC was determined for all the study subjects in triplicate by the chemiluminescent slot blot assay as previously described^{8, 9}. TC for each sample is reported as the ratio of the TC in the representative sample to a placental DNA control. The intra-sample mean coefficient of variation was 27%.

Statistical methods

Survival distributions were estimated using the Kaplan-Meier method and compared with log-rank tests. Subjects were censored if lost to follow-up or died prior to any detectable rise in PSA levels. Hazard ratios (HR) and 95% confidence intervals (CI) were determined using Cox proportional hazards models. Multivariate approaches were developed in a manual hierarchical approach based on the likelihood ratio test and changes in the magnitude of hazard ratios. Simple linear regression models and correlation coefficients were used for all correlations. Statistical analyses were performed using R statistical software version 1.14 from the R Foundation for Statistical Computing®.

RESULTS

TC and prediction of PSA recurrence

Of the 103 men who underwent radical prostatectomy, 71 (68.9%) men remained alive, 27 (26.2%) men died, 5 (4.8%) died due to prostate cancer, and 5 (4.9%) men were lost to follow-up over a mean follow-up period of 114 months (range 1–165). Table 1 lists the characteristics of the study population for all the study subjects, as well as a subset analysis of men who had a biopsy Gleason sum ≤ 6 . During the follow-up period, 46 (44.7%) of all study subjects had a PSA recurrence, which is in the range with previous reports⁴.

Univariate analysis of TC and PSA recurrence

Table 2 lists the univariate Cox proportional hazards models for TC and other pre-operative prognostic variables with respect to time (in months) to event of post-prostatectomy PSA recurrence for all study subjects and the subset of men with biopsy Gleason sum ≤ 6 . To determine a TC cutoff, the cohort was divided into quintiles, the survival interval for each group was calculated, groups with statistically indistinguishable survival intervals were combined and the process was repeated until only groups with significantly different survival intervals remained. The lowest quintile (<0.3) was used as the cutoff point for the TC variable, while other commonly utilized cutoffs were used for the other markers. When examining all

study subjects, significant associations were seen with low TC (HR=1.94; 95% CI:1.02–3.69, $p=0.04$), a pre-operative PSA value ≥ 10.0 ng/mL (HR=3.06; 95% CI:1.04–9.02, $p=0.04$), and biopsy Gleason sum ≥ 8 (HR=2.47; 95% CI:1.18–5.17, $p=0.02$) in predicting early PSA recurrence. In the subset analysis, TC was the only pre-operative variable that remained a significant predictor of early PSA recurrence. In this context, TC actually improved in predictive ability (HR=4.53; 95% CI:2.00–10.2, $p<0.01$).

Kaplan-Meier survival analysis of TC and PSA recurrence

For all subjects, Kaplan-Meier survival analysis for time to PSA recurrence following radical prostatectomy in regards to TC demonstrated significantly different PSA recurrence-free survival curves (survival rate for men with TC ≥ 0.3 of $56\% \pm 6\%$ and TC < 0.3 of $38\% \pm 11\%$; $p=0.04$, Figure 1). Alternatively, in the subset analysis of men with biopsy Gleason sum ≤ 6 disease, the PSA recurrence-free survival curves widened further for TC (survival rate for men with TC ≥ 0.3 of $65\% \pm 8\%$ and TC < 0.3 of $23\% \pm 12\%$; $p<0.01$, Figure 1). These models strongly support the hypothesis that low TC predicts early PSA recurrence. Specifically, low TC predicts early rise in PSA in men with low grade disease, helping to separate this clinically homogenous group into men with high and low risk of PSA recurrence post-prostatectomy prior to undergoing any treatment.

Multivariate analysis of TC and PSA recurrence

A multivariate Cox proportional hazards model for TC and the other pre-operative variables with respect to time (in months) to event of post-prostatectomy PSA recurrence for all study subjects and the subset of men with biopsy Gleason sum ≤ 6 is shown in Table 2. The association of low TC to post-prostatectomy PSA recurrence when adjusted for pre-operative variables (highest pre-operative PSA value and biopsy Gleason sum) was not statistically significant (HR=1.89; 95% CI:0.92–3.88, $p=0.08$) for all study subjects. However, in the subset analysis, the overall hazard for low TC (HR=6.62; 95% CI:2.69–16.3, $p<0.01$) became highly significant. There were no significant interactions or associations of low TC and the other prognostic markers in this study. No correlations were seen in regression models of TC compared to highest pre-operative PSA and biopsy Gleason sum (multiple $R^2=0.0018$, $p=0.68$ and multiple $R^2=0.00073$, $p=0.79$, respectively). When controlling for pre-operative PSA, low TC measured in biopsy samples significantly predicted early PSA recurrence, demonstrating a potential clinical role as a pre-operative prognostic marker.

COMMENT

In the present study, we show that TC measured in the prostate biopsy specimen predicts early PSA recurrence after radical prostatectomy. Men with low TC (< 0.3) were at significant risk of having an earlier rise in their PSA, suggesting recurrence of their disease despite definitive surgical treatment. This study confirms our previous work in which TC in prostatectomy tissue independently predicted PSA recurrence¹⁰, and extends the hypothesis to suggest TC measured in biopsy tissue, can be used as a predictive marker for radical prostatectomy outcome along with other established and clinically available pre-operative prognostic markers, such as PSA and biopsy Gleason sum. In this study, TC predicted recurrence in men who had low grade tumors better than in men with high grade disease. This unique predictive quality of TC could help patients faced with the challenge of weighing the risks and benefits of surgical treatment when they are diagnosed with low grade prostate cancer.

Telomeres are specialized nucleoprotein structures that protect the ends of eukaryotic chromosomes from degradation and recombination, and due to incomplete replication are shortened during each round of cellular replication. Shortened, dysfunctional telomeres are prone to chromosome fusion and breakage. In normal somatic cells this leads to timely p53-

dependent senescence and apoptosis^{12, 13}. In cancer cells, these mechanisms are inactivated, and if unchecked, the accumulation of these chromosomal aberrations is lethal. Consequently, stabilization of telomeres, through activation of telomerase, is essential for tumor progression^{14, 15}. The cause-and-effect relationship between dysfunctional telomeres and genomic instability implies that tumors with the shortest telomeres have the least stable genomes, and thus, have the greatest probability of containing cells capable of invasion, extravasation and metastasis. Likewise, tumors with the longest telomeres would be expected to have fewer genomic alterations, and therefore, lower probability of containing cells with the phenotypes associated with disease recurrence. Consistent with this view, numerous studies have associated reduced telomere length with poor clinical outcome or markers of disease progression¹⁵. Additionally, Joshua and colleagues recently demonstrated that short telomeres in biopsies containing high grade prostatic intraepithelial neoplasia or the surrounding stroma were indicative of a subsequent diagnosis of prostate cancer¹⁷.

Whether TC can predict significant morbidity or mortality from prostate cancer is not known. This study was not specifically designed to address clinical progression and survival. PSA recurrence may not be a valid surrogate end-point for radical prostatectomy outcome and disease-free survival¹⁸; however, the use of PSA recurrence makes a compelling argument to further investigate TC in this context.

Prior studies indicate that telomere shortening occurs in precursor lesions, preceding histological changes associated with complete malignant transformation^{19–21}. Likewise, in our previous study, TC in tumor tissue correlated to matched, histologically normal adjacent tissue suggesting a “field effect”, or “field cancerization” in the surrounding tissue¹⁰. The distance from the tumor tissue to which the field extends is not known or well characterized. Therefore, a biopsy core containing a vector sample of tissue with only a fraction of adenocarcinoma may be a mixture of normal tissue telomere lengths and shortened preneoplastic and/or neoplastic telomere lengths, inadvertently increasing or decreasing the observed TC ratio. The fact that we were able to discern a difference in outcome without microdissection, despite a possible “field effect”, attests to the robustness of this particular telomere DNA assay.

This study attempted to pre-define and include all men undergoing radical prostatectomy in a certain time period to reduce the potential limitations and biases of a retrospective analysis. The exclusion and lost-to-follow-up rate is extremely low, strengthening the validity of these results. Only a well-designed prospective study would completely resolve these limitations. In agreement with our previous studies^{10, 11}, this study continues to support the hypothesis that decreased telomere length induces genomic instability and increased mutation rates which, in turn, lead to more aggressive tumors with elevated growth rates and/or metastatic capability. Nonetheless, the mechanisms by which low TC confers risk of PSA recurrence cannot be determined by this clinical approach. Further clinical and basic science investigations should be pursued to address these issues.

CONCLUSIONS

Low TC measured in the prostate needle biopsy tissue predicts early likelihood of post-prostatectomy PSA recurrence in a retrospective analysis and in men with low grade disease (i.e. biopsy Gleason sum ≤ 6) is also independent of pre-operative PSA. This study indicates the potential clinical use of TC in prostatic biopsies as an independent prognostic marker for early PSA recurrence after surgical treatment.

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Biopsy TC Ratio

Figure 1.

Kaplan-Meier survival analysis with respect to PSA recurrence-free survival for groups determined by a telomere DNA content (TC) cutoff of 0.30 for all subjects (n=103) and for subjects with biopsy Gleason sum ≤ 6 (n=63). Tick marks indicate censored events.

Table 1

Characteristics of men undergoing radical prostatectomy for the study population.

	All Study Subjects	Subjects with Biopsy Gleason Sum ≤6
Patients (n)	103	63
Age at Diagnosis		
Mean (years)	63.6	63.4
Range	48–74	48–74
Follow-Up Time		
Mean (months)	114	115
Range	1–165	1–164
Biochemical Recurrence		
Yes	46 (44.7)	25 (39.7)
No	57 (55.3)	38 (60.3)
Time to PSA Recurrence		
Mean (months)	23	30
Range	0–122	1–122
Biopsy TC		
≥0.30	82 (79.6)	50 (79.4)
<0.30	21 (20.4)	13 (20.6)
Pre-Operative PSA (ng/mL)[†]		
<4.0	16 (15.5)	10 (15.9)
4.0–10.0	51 (49.5)	32 (50.8)
≥10.0	33 (32.0)	19 (30.2)
Biopsy Gleason Sum		
≤6	63 (61.1)	63 (100)
7	18 (17.5)	0 (0)
≥8	15 (14.6)	0 (0)
Undetermined [‡]	7 (6.8)	0 (0)
Pathologic Gleason Sum		
≤6	35 (34.0)	26 (41.3)
7	49 (47.6)	30 (47.6)
≥8	19 (18.4)	7 (11.1)
Organ Confined Disease		
No	42 (40.8)	28 (44.4)
Yes	61 (59.2)	35 (55.6)
Seminal Vesicle Invasion		
Absent	88 (85.4)	55 (87.3)
Present	15 (14.6)	8 (12.7)
Surgical Margin Involvement		
Absent	57 (55.3)	37 (58.7)

	All Study Subjects	Subjects with Biopsy Gleason Sum ≤ 6
Present	46 (44.7)	26 (41.3)

Unless otherwise noted, data are presented as number of patients with percentages in parentheses

[†]Three men were missing documented pre-operative PSA values.

[‡]Limited tumor tissue in the biopsy sample prohibited the pathologist from assigning a Gleason sum.

Table 2

Univariate and multivariate Cox proportional hazards model demonstrating low telomere DNA content (<0.3) predicts early likelihood for PSA recurrence adjusted for pre-operative variables for the study population.

Univariate Analyses			Multivariate Analyses	
	Hazard Ratio (95% CI)	p=	Hazard Ratio (95% CI)	p=
All Study Subjects (n=93[†])				
Biopsy Telomere DNA Content				
≥0.30	1.00		1.00	
<0.30	1.94 (1.02–3.69)	0.04	1.89 (0.92–3.88)	0.08
Pre-Operative PSA (ng/mL)				
<4.0	1.00		1.00	
4.0 – 10.0	2.13 (0.73–6.20)	0.17	2.35 (0.68–8.23)	0.18
≥10.0	3.06 (1.04–9.02)	0.04	3.31 (0.95–11.6)	0.06
Biopsy Gleason Sum				
≤6	1.00		1.00	
7	1.49 (0.69–3.21)	0.75	1.19 (0.53–2.70)	0.67
≥8	2.47 (1.18–5.17)	0.02	1.79 (0.79–4.05)	0.16
Subjects with biopsy Gleason Sum ≤6 (n=61[†])				
Biopsy Telomere DNA Content				
≥0.30	1.00		1.00	
<0.30	4.53 (2.00–10.2)	<0.01	6.62 (2.69–16.3)	<0.01
Pre-Operative PSA (ng/mL)				
<4.0	1.00		1.00	
4.0 – 10.0	1.20 (0.33–4.38)	0.75	2.30 (0.60–8.80)	0.22
≥10.0	2.20 (0.60–7.99)	0.23	4.08 (1.06–15.7)	0.04

[†] Subjects with incomplete or missing data were excluded from the analysis.